# Foraging and Nesting Behavior of *Osmia lignaria* (Hymenoptera: Megachilidae) in the Presence of Fungicides: Cage Studies

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J. Econ. Entomol. 101(3): 647–653 (2008)

**ABSTRACT** During orchard pollination studies in California, we observed dramatic changes in nesting and foraging behavior of *Osmia lignaria* Say (Hymenoptera: Megachilidae) after sprays with tank mixtures containing fungicides. A characteristic pattern of postspray events observed includes erratic behavior and interrupted foraging and nesting activity for several days. In an effort to determine whether fungicidal sprays were disruptive to bee foraging and thus to pollination, we exposed *O. lignaria* females nesting in field cages planted with lacy scorpionweed, *Phacelia tanacetifolia* Benth (Hydrophyllaceae), to selected spray mixtures normally encountered in California orchard production systems: iprodione (Rovral), propiconazole (Orbit), benomyl (Benlate), and captan (Captan 50 WP); the surfactant Dyne-Amic, alone and mixed with Rovral; and the tank mixture IDB (Rovral + Dyne-Amic + the foliar fertilizer Bayfolan Plus). An additional cage sprayed with an equal volume of water acted as control, and a cage sprayed with the insecticide dimethoate as a toxic standard. For each female *O. lignaria*, we recorded time spent inside the nest depositing pollen-nectar loads, foraging time, cell production rate, and survival. All females in the dimethoate treatment died postspray + 1 d. Before death, some of these females behaved similarly to our previous orchard observations. A high proportion of females in the IDB cage were inactive for a few hours before resuming normal foraging and nesting activity. No lethal or behavioral effects were found for any of the other compounds or mixtures tested. Our results indicate that the fungicide applications that we tested are compatible with the use of *O. lignaria* as an orchard pollinator.

**KEY WORDS** orchard pollination, side effects, behavioral effects, sublethal dosages

The solitary bee *Osmia lignaria* Say (Hymenoptera: Megachilidae) has been developed as a commercial orchard pollinator in North America (Torchio 1985, 2003; Bosch and Kemp 2001, Bosch et al. 2006). *O. lignaria* is a univoltine, spring-flying species. Females build multicelled nests in preestablished cavities such as beetle borings in dead wood, or, in populations managed for orchard pollination, artificial nesting materials (wood blocks, reeds, and cardboard tubes). *O. lignaria* females collect mud to build partitions between individual cells and pollen-nectar to provision each cell. Only one egg is laid per cell. In field conditions, females build and provision an average of 0.5–1 cells per day (Bosch and Kemp 2001). Pollen–nectar foraging trips last 10–15 min and mud-collecting trips last 2–3 min. Both pollen–nectar deposition and mud deposition inside the nest last ≈ 2 min. Upon depositing a pollen–nectar or a mud load, females immediately start a new foraging trip. When the nest is complete, females collect mud again to cap the entrance, and search for another nesting cavity in which to start a new nest. Throughout her life, a female may build an average of four nests, but a female never works simultaneously on more than one nest (Bosch and Kemp 2001). Throughout the nest building and provisioning process, females mark their nest entrance with an abdominal secretion, probably from the Dufour's gland (Guédot et al. 2006). Upon returning from a foraging trip, females usually enter their nesting cavity with no or little hesitation. If the nest marking is removed, females do not recognize their nesting cavity, and they start inspecting adjacent nesting cavities (Guédot et al. 2006).

In 2001, during commercial cherry pollination studies in California, we observed dramatic behavioral changes in nesting *O. lignaria* females after chemical sprays applied during full bloom (Ladurner et al. 2005). These sprays were applied by producers, and they consisted of tank mixtures of the fungicides benomyl, captan, iprodione, or propiconazole with surfactants and foliar fertilizers. Sprays were conducted at

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night, when *O. lignaria* females were inactive inside their nesting cavities. To avoid direct spray on the bees or the nesting materials, shelters were covered during the sprays. The morning after the sprays, homing, nesting, and foraging behaviors of well-established females were visibly impaired. Upon returning from their first morning foraging trip, females hovered in front of the nesting sites and repeatedly entered and exited various nesting cavities, as if they could not recognize their own nest. Subsequently, females spent an inordinately long time inside a nesting cavity, completely forsaking pollen–nectar collection despite good weather conditions. Cell production was completely interrupted for entire days. In some cases, females resumed nesting activity 3–4 d after the sprays. In other cases, they progressively disappeared from the nesting site. Despite extensive searching, no dead *O. lignaria* were found by the nesting sites or under the sprayed fruit trees. In nonsprayed orchards, females entered their nesting cavity with no or little hesitation, and they did not halt cell provisioning for long periods unless weather conditions were unfavorable for foraging. By the end of the flowering period, cell production in three sprayed orchards was 108.3 ± 88.2 (mean ± SE) compared with 10,012 in one nonsprayed orchard blooming at the same time in the same area (J.B., W.P.K., and E.L., unpublished data). Although honey bees and mason bees have different foraging and nesting behaviors, it is interesting to note that some of the symptoms observed in our California field studies coincide with symptoms detected in honey bees exposed to the systemic insecticide imidacloprid. In various studies, honey bees fed sublethal doses of imidacloprid in sucrose solution showed decreases in foraging activity, olfactory discrimination, learning performance, and homing ability (Curé et al. 2001, Decourtye et al. 2001, Bortolotti et al. 2003, Medrzycki et al. 2003). As in our field observations with *O. lignaria*, these behavioral responses were temporary, and although no conclusive results have been reached, exposure to imidacloprid has been related to colony collapse disorder (also called disappearing disease), a syndrome characterized by a depopulation of the colony with little or no buildup of dead bees in or around the hives (Bortolotti et al. 2003).

In an effort to determine whether fungicide sprays such as those conducted during our California studies could be disruptive to *O. lignaria* foraging and nesting activity, and thus pollination performance, we initiated a series of laboratory and field studies. In 2002, we investigated the acute and delayed toxicity of the fungicides benomyl, captan, iprodione, propiconazole, and neem oil (also used as insecticide) to *O. lignaria* and *Apis mellifera* L. under controlled laboratory conditions (Ladurner et al. 2005). Captan severely affected survival of *O. lignaria*, and at 7 d from exposure the oral HQ (Hazard Quotient; ratio between the highest recommended field rate in grams of active ingredient (AI)/ha and the contact and oral $LD_{50}$ values in micrograms (AI) per bee reached 202, well above the threshold of 50, the level considered hazardous to bees (Cluzeau 2002; EPPO 1993, 2001). HQ in bees are usually calculated using 24 h $LD_{50}$ values, but the toxic effect of Captan to *O. lignaria* was only evident several days after treatment (Ladurner et al. 2005). Orally administered propiconazole resulted in acute and delayed toxicity to *O. lignaria*, but the oral HQ was only 4. The other fungicides tested showed no toxic effect on *O. lignaria.* However, sublethal or behavioral effects are usually difficult to detect in standard laboratory toxicity tests (Stark et al. 1995, Kovács et al. 1999, Lewis et al. 2001, Cluzeau 2002, Pham-Delègue et al. 2002, Thompson 2003, Desneux et al. 2007). Effects on associative learning may be assessed through proboscis extension response and maze tests in the laboratory (Decourtye et al. 2004; Desneux et al. 2007), but effects on homing ability and foraging behavior such as those observed in our orchard trials need to be studied under field or semifield conditions (Vandame et al. 1995, Bortolotti et al. 2003, Taséi and Dinet 1981, Fell et al. 1983, Mayer and Lunden 1999, Schmuck et al. 2003, Colin et al. 2004). For this reason, cage and field studies are recommended as a more reliable method to detect this type of behavioral effects (EPPO 1993, 2001). Standard laboratory tests may also fail to detect potential synergistic effects between plant protection products and spray adjuvants (Wallner 1999, Cluzeau 2002, Pham-Delègue et al. 2002, Thompson 2003). Pesticide hazard may vary depending on the presence of adjuvants in the tank mixture, with different bee species expressing different responses (Ross and Harvey 1981, Mayer et al. 1987, Mayer and Lunden 1999). Some adjuvants alone have shown acute toxicity effects on *A. mellifera* in the laboratory (Goodwin and McBrydie 2000). In this article, we report on cage studies designed to investigate the possible side effects of fungicide sprays (some in combination with a surfactant and a foliar fertilizer) on nesting *O. lignaria* females. Our objective was to identify one or more products or product combinations that resulted in dramatic behavioral changes similar to those observed during our pollination studies of 2001.

## Materials and Methods

Studies were conducted during 2003 and 2004 in an experimental field drilled with lacy scorpionweed, *Phacelia tanacetifolia* Benth (Hydrophyllaceae), in Logan, UT. *P. tanacetifolia* is a common pollen–nectar source for *O. lignaria.* No pesticides were applied to either the seeds or the emerged crop. With the onset of bloom, study plots of 37.2 $m^2$ each were confined within the field with anti-aphid screen cages (6.2 by 6.2 by 2 m; 1- by 1-mm mesh size). An *O. lignaria* nesting shelter was placed in the center of each cage. Shelters consisted of wooden boxes (30 by 30 by 35 cm) with the front side open and facing east, held 1.2 m above the ground on metal fence posts. Each shelter was provided with one nesting unit, consisting of a solid wood block (15 by 15 by 16.5 cm) with paper straws (7.5 mm in diameter) inserted in 49 15-cm holes drilled into the block. To facilitate observations, nesting cavities were numbered with white grease pencils.

Each cage had a mud source consisting of a trench, which was kept moist throughout the study period.

When the *P. tanacetifolia* was in full bloom (May–June), male and female *O. lignaria* from populations reared at the Logan Bee Biology and Systematics Laboratory were incubated at 22°C until emergence. After emergence, females were chilled at 4°C and individually marked in the laboratory with enamel colors. From 15 to 18 males and 10–14 individually marked females were released in each cage. Two days after release, when mating activity had ceased, males were removed from the cages. Once at least five females per cage had established (selected a nesting hole and commenced foraging and nesting activities), each cage was randomly assigned to a treatment. All treatments were applied at the highest recommended field rate for stone fruits with hand-held sprayers, at a rate of 1021 liters of water per ha. *O. lignaria* females were allowed to forage on the treated plots for 4–5 d, after which cages were opened and the nesting units brought to the laboratory. Temperature and relative humidity inside the cages were recorded throughout the study.

**Toxic Standard.** To verify that females were exposed to the pesticides sprayed on the flowers, we conducted a toxic standard treatment on 25 May 2003. We sprayed a cage with the standard reference insecticide dimethoate (Dimethoate 267, FMC Corporation; active ingredient, dimethoate 30.5%) at twice the highest recommended field rate (recommended rate 1.75 liters formulated product (henceforth f.p.)/ha, i.e., 0.56 kg [AI]/ha). We used twice the field rate because dimethoate at a field rate of 0.450 kg (AI)/ha did not cause adverse effects to alfalfa leafcutting bee, *Megachile rotundata* F., another megachilid bee species (Johansen et al. 1983). Sprays were applied in the evening from 2130 to 2230 h, after *O. lignaria* females had ceased foraging.

**Experiment 1.** On 27 May 2003, four cages were sprayed each with a different fungicide. The fungicides and the applied rates were as follows: 1) iprodione (Rovral 50 WP, Rhône Poulenc, [AI] iprodione 50%), 2.24 kg f.p./ha; 2) propiconazole (Orbit, Novartis, [AI] propiconazole 41.8%), 0.3 liters f.p./ha; 3) benomyl (Benlate 50 WP from DuPont, [AI] benomyl 50%), 2.24 kg f.p./ha; and 4) Captan (Captan 50 WP, Helena Chemical Company [AI] captan 48.9%), 4.48 kg f.p./ha. A fifth cage was sprayed with an equal volume of water, thus acting as control. Sprays were applied in the evening.

**Experiment 2.** On 4 June 2003, one cage was sprayed with the surfactant Dyne-Amic, a 99% proprietary blend of nonionic organosilicone surfactants and methyl-esters of C16–C18 fatty acids (highest recommended use rate 0.75%, vol:vol). An additional cage was sprayed with the fungicide iprodione (Rovral, 2.24 kg f.p/ha) in tank mixture with the surfactant Dyne-Amic. Finally, a third cage was sprayed with an equal volume of water, thus acting as control. Sprays were applied in the evening.

**Experiment 3.** On 4 June 2004, one of two cages was sprayed with a tank mixture (henceforth IDB) of the fungicide iprodione (Rovral, 2.24 kg f.p/ha), the surfactant Dyne-Amic (0.75%, vol:vol), and the foliar fertilizer Bayfolan Plus, an inorganic liquid nutrient for foliar feeding (recommended use rate 5%, vol:vol). This specific mixture was used in one of the California orchards in which we observed non-normal nesting behavior of *O. lignaria.* The second cage was sprayed with an equal volume of water (control). Sprays were applied in the evening. On 7 June 2004, we repeated both treatments in two additional cages, but sprays were applied in the early morning, before bee foraging.

**Data Collection.** In each cage, on day 0 (day of treatment for evening applications; day before treatment for morning applications), and on days 1, 2, and 4, we recorded the number of actively nesting females. On each of these days, observations on the behavior of each female were recorded on audio tapes for 1 h (≈1100–1200 hours). Tapes were then transcribed to determine, for each female, the mean time spent inside the nest depositing pollen and nectar loads (in-nest time), and the mean time spent outside the nest foraging for pollen and nectar (foraging time). To assess for potential bee mortality, every evening throughout the study period, we inspected nesting cavities with a flashlight, and we counted the number of females (*O. lignaria* females spend the night in their nesting cavity). During these evening counts, paper straws containing females were removed with forceps, and nest progression (evidenced by placing the straw against a flashlight) was marked and dated on each straw. This provided a precise measure of the number of brood cells produced per day by each female (cell production rate).

**Statistical Analysis.** Due to the limited number of cages and observers available (nesting and foraging behavior had to be monitored simultaneously in each cage), it was not possible to replicate our study at the cage level. Although we are aware of the limitations of this design (Hurlbert 1984), we think it does not bias our conclusions for two reasons. First, we were not trying to detect subtle differences in nesting and foraging behavior, but rather to assay for dramatic behavioral changes, such as those observed in our field studies. Second, our objective was to screen as many chemicals or combinations of chemicals as possible in an attempt to pinpoint one or more treatments eliciting the type of response observed in our field studies. Thus, we decided to invest the limited number of cages and observers to screen as many treatments as possible, rather than replicate treatments that clearly failed to reproduce the response observed in our field studies (see results). We analyzed our data in a split-plot design, with each cage as a plot and the individual bees as subplots. For each female, in-nest times, foraging times and cell production rates on days 1, 2, and 4 were standardized by dividing these measures by the measure recorded on day 0 (before the applications). To address normality and homoscedasticity, standardized values were log-transformed. Transformed mean in-nest times, foraging times, and cell production rates on the different observation days (within-subjects factor) were then compared across treatments (be-

**Table 1. Summary of repeated measures ANOVA on the effect of treatment and day on in-nest time, foraging time, and cell production rate**

| Exp | Treatment | Variable | Effect | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Treatment | | Day | | Treatment × day | |
| | | | df | *F* value | df | *F* value | df | *F* value |
| 1 | Iprodione, propiconazole, benomyl, captan, control | In-nest time | 4, 9 | 0.72 | 2, 18 | 15.50** | 8, 18 | 4.49* |
| | | Foraging time | 4, 9 | 1.41 | 2, 18 | 2.10 | 8, 18 | 0.93 |
| | | Cell production rate | 4, 21 | 0.53 | 3, 63 | 0.35 | 12, 63 | 1.40 |
| 2 | Dyne-Amic, iprodione + Dyne-Amic, control | In-nest time | 2, 4 | 0.12 | 2, 8 | 0.11 | 4, 8 | 1.43 |
| | | Foraging time | 2, 4 | 1.15 | 2, 8 | 1.56 | 4, 8 | 0.62 |
| | | Cell production rate | 2, 18 | 0.13 | 3, 54 | 1.16 | 6, 54 | 1.20 |
| 3 | IDB, control (evening application) | Cell production rate | 1, 11 | 0.14 | 3, 33 | 15.66** | 3, 33 | 1.25 |
| | IDB, control (morning application) | In-nest time | 1, 5 | 0.71 | 2, 10 | 3.77 | 2, 10 | 2.19 |
| | | Foraging time | 1, 5 | 4.40 | 2, 10 | 0.55 | 2, 10 | 0.97 |
| | | Cell production rate | 1, 19 | 1.68 | 3, 57 | 1.05 | 3, 57 | 0.54 |

*, $P < 0.05$; **, $P < 0.0001$.

tween-subjects factor) using repeated-measures analysis of variance (ANOVA). During data collection, some females were building mud structures (cell partitions or nest caps) or selecting a new nesting cavity. Thus, pollen–nectar foraging and in-nest deposition times could not be measured for these females. Only females for which foraging and in-nest times could be recorded on every observation day were included in the analyses.

## Results

**Toxic Standard.** Ten females were actively nesting on day 0 in the toxic standard dimethoate cage. The morning after the spray (day 1), some of these females were still alive. A few were observed entering and exiting various nesting cavities, whereas others were inactive in their nesting cavities. Only one female was observed carrying pollen on her scopa, but none of the females were actively nesting. These behaviors were reminiscent of our field observations in California. In the afternoon all females were dead. This result verified that *O. lignaria* females were effectively exposed to the sprays delivered to the plants.

**Experiment 1 (Treatments: Iprodione, Propiconazole, Benomyl, Captan, and Water Control).** Ambient conditions inside the cages were favorable to the foraging activity of *O. lignaria* females throughout the experiment. We observed no treatment effects on the survival of *O. lignaria* females (7–9 per cage): populations remained constant throughout the experiment. No significant differences among treatments were observed for any of the three variables measured (in-nest time, foraging time, and cell production rate) (Table 1). There was a significant day effect and a significant treatment × day interaction for in-nest time (Table 1), which tended to increase over time, especially in the benomyl treatment (Fig. 1). Samples sizes were greater for cell production than for the other two variables, because some females were selecting a new nesting cavity and/or building nest structures, and thus collecting mud instead of pollen–nectar during the observation periods. As a consequence, we could measure cell production rate, but not in-nest and pollen–nectar foraging times for these females.

**Experiment 2 (Treatments: Iprodione + Dyne-Amic, Dyne-Amic, and Water Control).** Ambient conditions did not hinder the foraging activity of *O. lignaria*, and the number of nesting *O. lignaria* females (7–9 per cage) remained constant throughout the experiment. The effects of day and treatment on the three variables analyzed, and treatment × day interactions were not significant (Table 1).

**Experiment 3 (Treatments: Iprodione + Dyne-Amic + Bayfolan Plus, and Water Control).** Sprays did not affect the survival of *O. lignaria* females (8–14 per cage). During the first trial (evening application) ambient conditions hindered the foraging activity of *O. lignaria* females, especially on days 3 and 4. For this reason, we could not record sufficient in-nest and foraging times. As a consequence of inclement weather, there was a substantial cell production drop, and a significant day effect emerged (Table 1). The treatment × day interaction was not significant. Weather was fair throughout the second trial of this

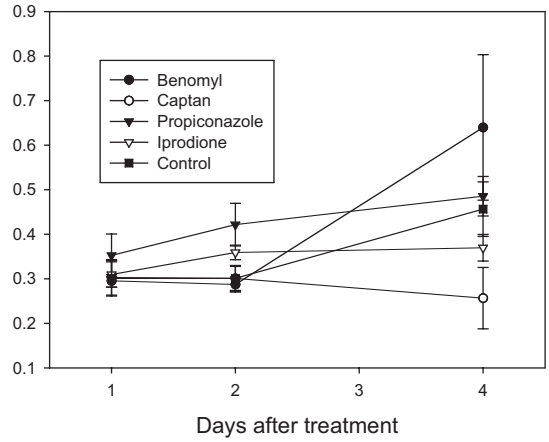


**Fig. 1.** Standardized mean ± SE in-nest time in cages treated with benomyl, captan, propiconazole, iprodione, and water (control).

experiment (morning application). In the morning of day 1, noticeably fewer females (four of 12 established females; 33.3%) were actively nesting in the treated cage compared with the control cage (13 of 14 established females; 92.9%). However, by the afternoon of day 1, all females were actively nesting in both cages. Differences in in-nest time, foraging time, and cell production rate between treatments and among days were not significant (Table 1). Treatment × day interactions were not significant.

## Discussion

Various species of *Osmia* have been developed as manageable pollinators of orchard and berry crops in various parts of the world (Bosch and Kemp 2002, Bosch et al. 2007). *Osmia cornifrons* (Radoszkowski) is the most widely used commercial orchard pollinator in Japan (Maeta 1990). As with honey bees and other pollinators managed for crop pollination, *Osmia* populations are routinely exposed to fungicide sprays on the assumption that fungicides are harmless to bees. However, honey bee losses after fungicide sprays have been repeatedly reported in various countries (Brasse 2001, Oomen 2001, Flechter and Barnett 2003, Rivera et al. 2003). In our study, no significant treatment effects were found on any of the three behavioral variables analyzed and for any of the fungicide treatments tested. A significant treatment × day interaction was recorded only for in-nest time in experiment 1. However, differences in in-nest time between benomyl and other treatments were very small, and in no way comparable with the dramatic changes observed in our California field studies. In none of the cages did we observe females entering different nesting cavities in quick succession, or staying inside a nesting cavity for abnormally long periods. More importantly, cell production was never affected by the sprays. Thus, all the products or product combinations tested failed to reproduce the drastic changes observed in commercial orchards.

Only two pieces of evidence obtained in this study are partially consistent with the observations made in our 2001 field studies. First, after the morning IDB application (but not the evening application) of experiment 3, some females temporarily suspended their nesting activity. However, this effect lasted only a few hours, compared with several days in the IDB-sprayed California orchard in 2001. Second, in the cage treated with the insecticide dimethoate, females behaved erratically in the hours previous to their death. Several factors (see below) may have contributed to the discrepancy between our 2001 field observations and the results of our cage studies.

*Tank Residues.* Pesticide residues are not uncommon in commercial spray tanks (Flechter and Barnett 2003), and they may have contaminated spray mixes used in the California orchards in which we observed non-normal nesting behavior. At sublethal doses, insecticides may elicit behavioral responses similar to those we observed in 2001. For example, field applications of Naled (Dibrom) did not induce mortality in populations of the alfalfa leafcutting bee, but they caused cell production to plummet (Torchio 1983). Honey bee foraging intensity decreased during 1 or 2 d after exposure to thiacloprid (Schmuck et al. 2003). In another study, caged honey bees expressed no unusual levels of mortality, but they seemed disoriented and hesitant when returning to their hive after imidacloprid and fenitrothion applications (K. Wallner, personal communication). Sublethal insecticide residues also could interact synergistically with fungicides (Pilling and Jepson 1993, Meled et al. 1998, Belzunces et al. 2001, Schmuck et al. 2003, Thompson and Wilkins 2003). A potential effect of insecticide residues is further supported by our observations of *O. lignaria* females temporarily having difficulty in recognizing their nest in the dimethoate-treated cage. Tank residue levels can be high enough to reach concentrations likely to result in toxic effects (Flechter and Barnett 2003).

*Field Rate.* Fungicide doses applied in California orchards during our field studies may have been higher than doses used in our study. This explanation is not supported by our previous laboratory studies (Ladurner et al. 2005), in which we observed no acute contact or oral toxicity effects of fungicides, even at high rates. However, as noted before, behavioral effects may be difficult to infer from non-nesting females, maintained in a reduced space under highly artificial laboratory conditions.

*Crop Species.* During our California field studies, sprays were applied to cherry (*Prunus* spp.) trees, whereas our cages were planted with *P. tanacetifolia.* Pesticide absorption of pesticides by plant tissues, and thus the exposure of the pesticide to pollinating insects, varies with plant species (Wallner 1994). Fungicide residues in nectar significantly varied among plant species exposed to the same treatment, even when samples were collected a few hours after treatment application (Schur and Wallner 2000, Pistorius and Wallner 2006). This explanation is not supported by our laboratory results (Ladurner et al. 2005), but, as mentioned, behavioral effects related to foraging and behavioral activity may be difficult to detect in non-nesting bees under laboratory conditions.

This study and our previous laboratory results (Ladurner et al. 2005) indicate that, for the range of compounds and tank mixes considered, and following label recommended procedures, fungicide sprays are compatible with the use of *O. lignaria* as an orchard pollinator. Nevertheless, further field research is necessary to elucidate the precise reasons for the nonnormal nesting behavior observed in our California studies. Future research should be directed toward detecting insecticide residues in commercial spray tanks, testing for behavioral effects on *O. lignaria* exposed to low-dose insecticide treatments, and toward the possible synergistic effects between fungicides and insecticide residues.

## Acknowledgments

Special thanks to S. Kalaskar, C. Guédot, G. Trostle, S. Crouzet, C. Gorrell, E. Klomps, and all the USDA–ARS, Bee

Biology and Systematics Laboratory staff for help throughout the study. We are grateful to K. Wallner (Hohenheim University) and D. Babendreier (Agroscope FAL Reckenholz) for comments on a draft of the manuscript. This study was partially supported by a Ph.D. scholarship from the University of Bologna to E.L.

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*Received 20 February 2007; accepted 8 June 2007.*